Integrating Liver Toxicogenomics with Clinical Pathology, Histopathology and Drug Metabolism Data in Preclinical Studies

Patrick Wier, Safety Assessment

Goals for Integration of Toxicogenomic Data

- Gene expression measurements highlight affected cellular pathways/processes
  - Opportunity to predict pathology
  - Can suggest modes of toxicity and possible "off-target" activities
- Provides another dimension to preclinical information used to characterize the compound
- To enhance, not supersede, well-established toxicological parameters

www.fda.gov/oc/initiatives/criticalpath/whitepaper.pdf, March 2004

-

"Often, developers are forced to use the tools of the last century to evaluate this century's advances"

"A new product development toolkit….is urgently needed to improve predictability and efficiency along the critical path"

Strategy for Integration of Toxicogenomic Data

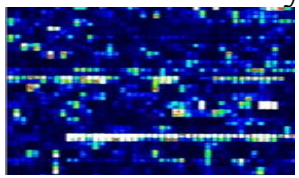
- Identify transcript changes in rat liver
  - associated with hepatotoxicity manifestations
  - suggestive of known modes of action
- Create robust assay methodology to support ca. 100 candidate studies/year without these data being rate limiting
- Provide reference knowledge based on previously characterized compounds

Hepatotoxicity Knowledge Base (HTKB)

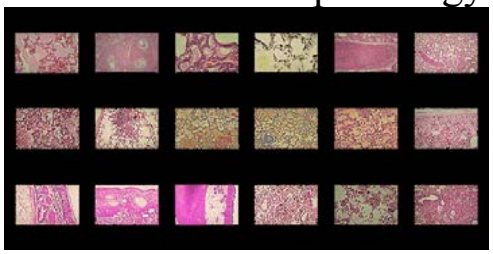
Input: Reference Data Base

Reference compounds

Liver Microarray



Clinical & Histopathology

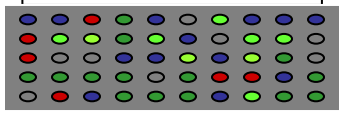


Analysis: Teams & Technology

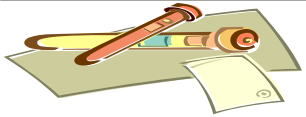
Output: Practical Preclinical & Clinical Applications

Liver Tox Target Panel

Liver Tox Gene Panel



Liver Tox Biomarkers



Liver Tox Candidate SNPs

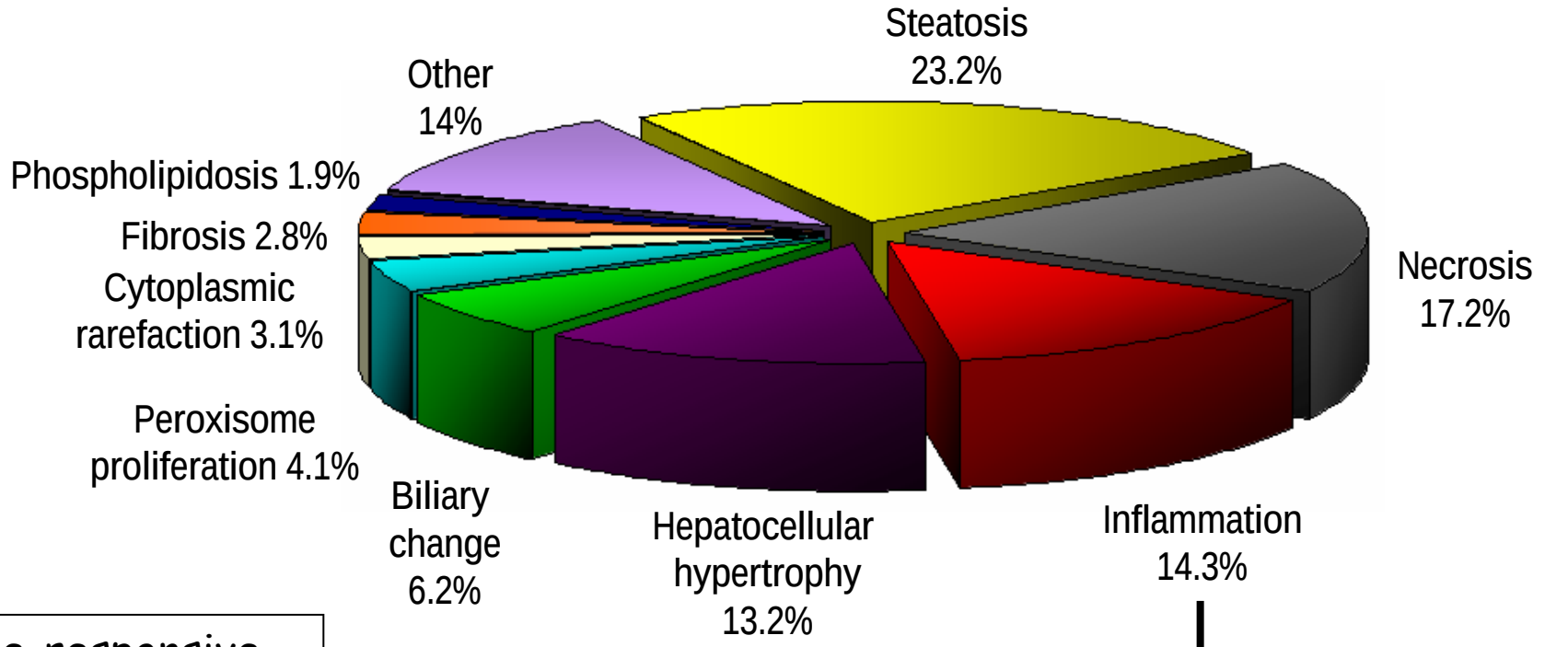
Lead Optimiz ⇒	*Cand Select* ⇒	⇒ *Drug Development* ⇒

Building the Reference Data

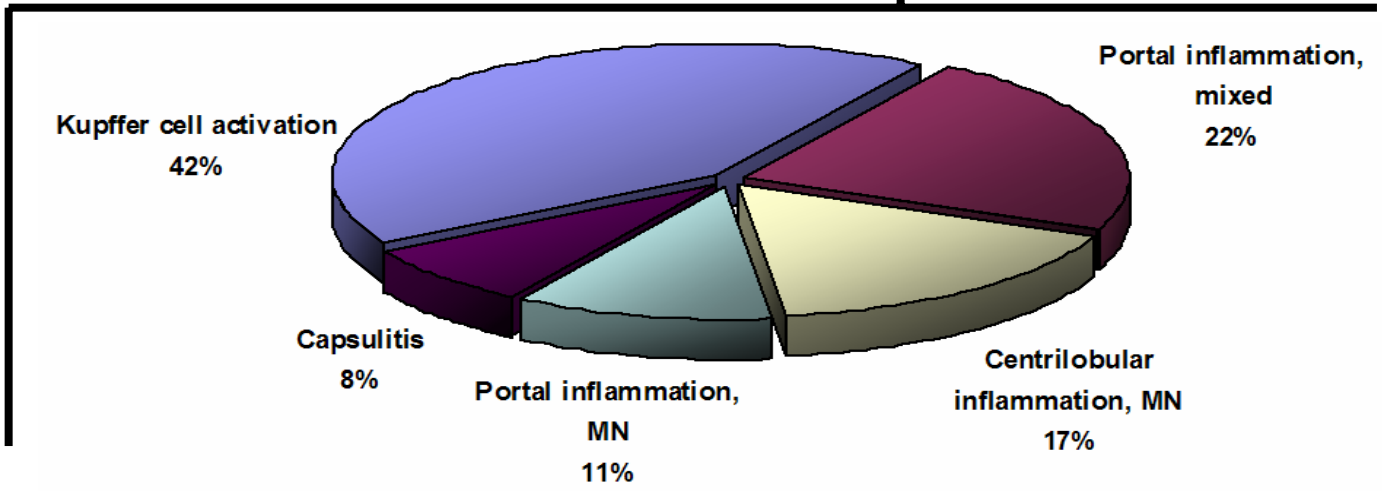
- Select known hepatotoxicants
  - representing a variety of hepatotoxicity manifestations
    - fibrosis, phospholipidosis, apoptosis, necrosis, cholestasis, biliary hyperplasia, peroxisome proliferation, acute phase response
  - representing a variety of hepatotoxicity mechanisms
    - free radical generation, membrane damage, covalent binding, altered lipid egress/mobilization, cytokine release, MMPT, hypoxia, mitogenesis, oxidative stress, impaired bile acid secretion, Kupffer cell activation, xxR-type induction
  - representing a variety of chemical and pharmacological classes
  - also including excipients, dietary modifications, and compounds without hepatotoxicity

>170 treatments in all

HTKB Data: Clinical & HistoPath Findings

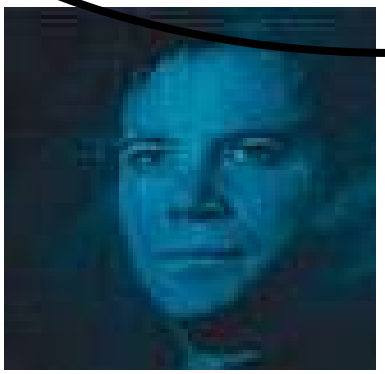


Dose-responsive increases in mean ALT in 34% of treatments
49%: > 3-fold
27%: 2 to 3-fold
24%: 1.5 to 2-fold



Data Analysis: The HTKB Mind-Meld

- ✓Is analysis linked to issue of value?
  - ✓Must interface with database…
- ✓Are the cohorts sound?
  - ✓Statistically sound analysis?
- ✓Confounding effects?
  - ✓How to define specificity?
- ✓Do the genes make sense?
  - ✓Gene annotation



Safety Assessment Buddy

Bio Informatics Buddy

HepatoTaq©: Rat Liver Toxicity Gene Panel

16 specifically focused subpanels

Manifestations of Hepatotoxicity

- Hepatic Fibrosis
- Hepatic Phospholipidosis
- Hepatocellular Apoptosis
- Zonal Hepatocellular Necrosis
  - Hepatocellular Cell Cycle
- Cholestasis
- Biliary Hyperplasia
- Hepatic Perox Proliferation
- Acute Phase Response

Modes of Hepatotoxicity

- Glutathione Depletion
- Lipid Peroxidation/ Mitochondrial Dysfunct
- Reactive Metabolites
- Drug Met Enzyme Modulation
  - AhR-type inducer
  - PXR-type inducer
  - CAR-type inducer
- Increased Hepatic Thyroid Hormone Clearance

- Measured with TaqMan™ microfluidic technology
  - faster, more quantitative, more economical, & more facile reporting than micro arrays

How to present & interpret HepatoTaq© data?

Ask toxicologists to think like toxicologists

Signal?

Consider relative to controls and biological variation

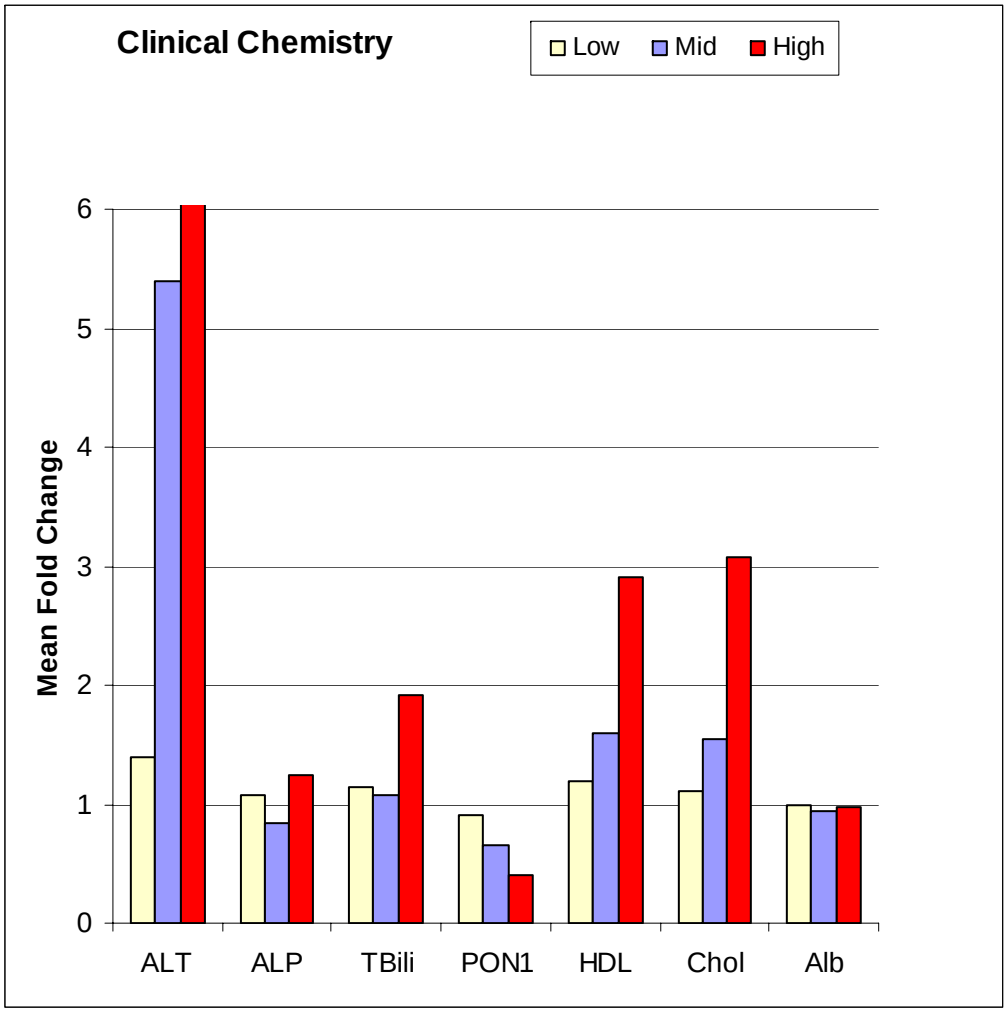
Dose-response?

Individual versus Group response?

(Group sizes (n=4) in practice too small for stats)

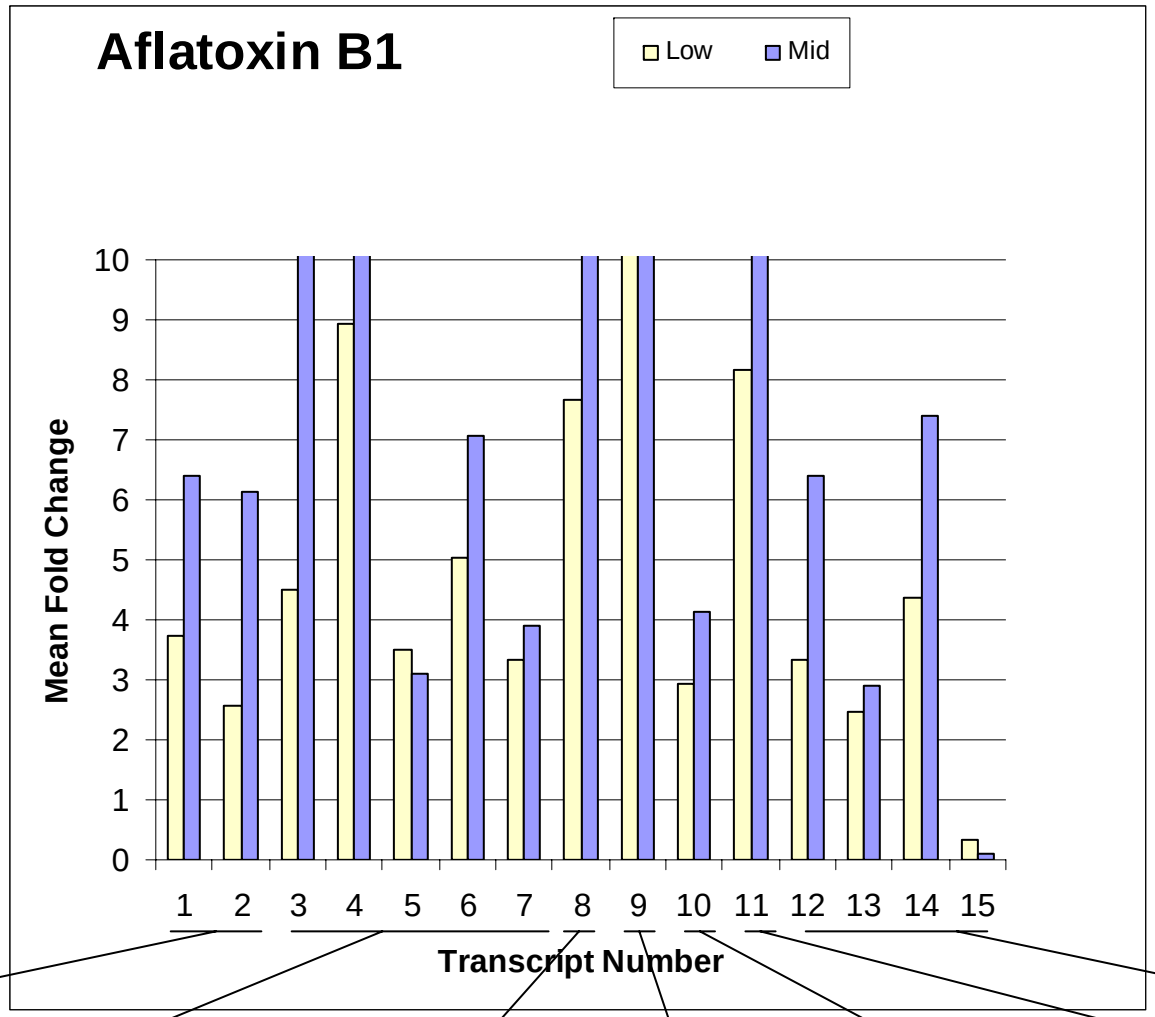
Perspective?

breadth of effects?
comparator compounds?



Hepatic Fibrosis Subpanel

Fibrous tissue deposition in response to cell death & mediators from activated stellate cells, fibroblasts, myo-fibroblasts



collagen component

Stellate Cell activation

↓ collagen proteolysis

degrades elastin

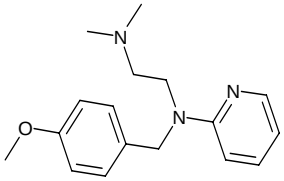
conn. tissue remodeling

↑ expression in cirrhosis

?

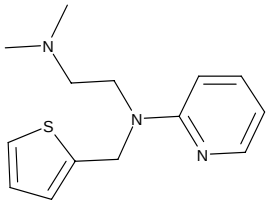
- AB1, DMF: subpanel dose-response preceded histology
- DMN, CCL4: subpanel (+) without histol. in 4d
- No false (+)

Reactive Metabolite Subpanel



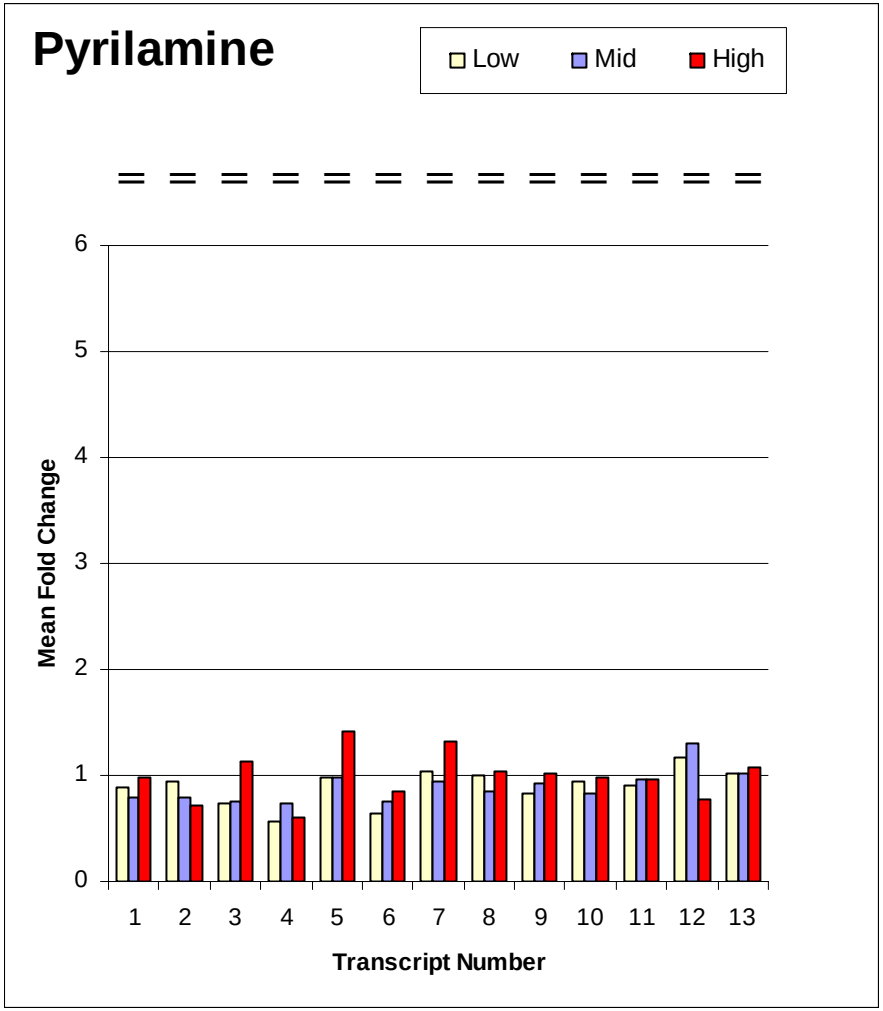
Pyrilamine

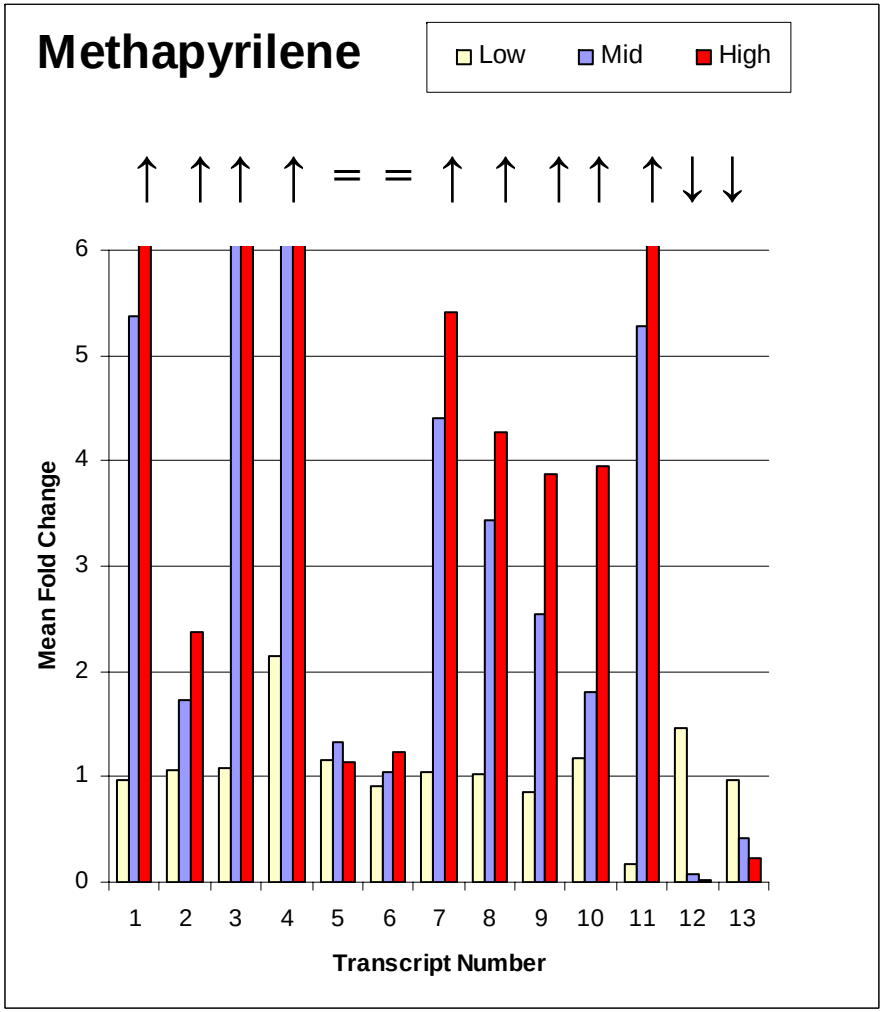
Non-hepatotoxic analog



Methapyrilene

HC necrosis, reactive metabolite





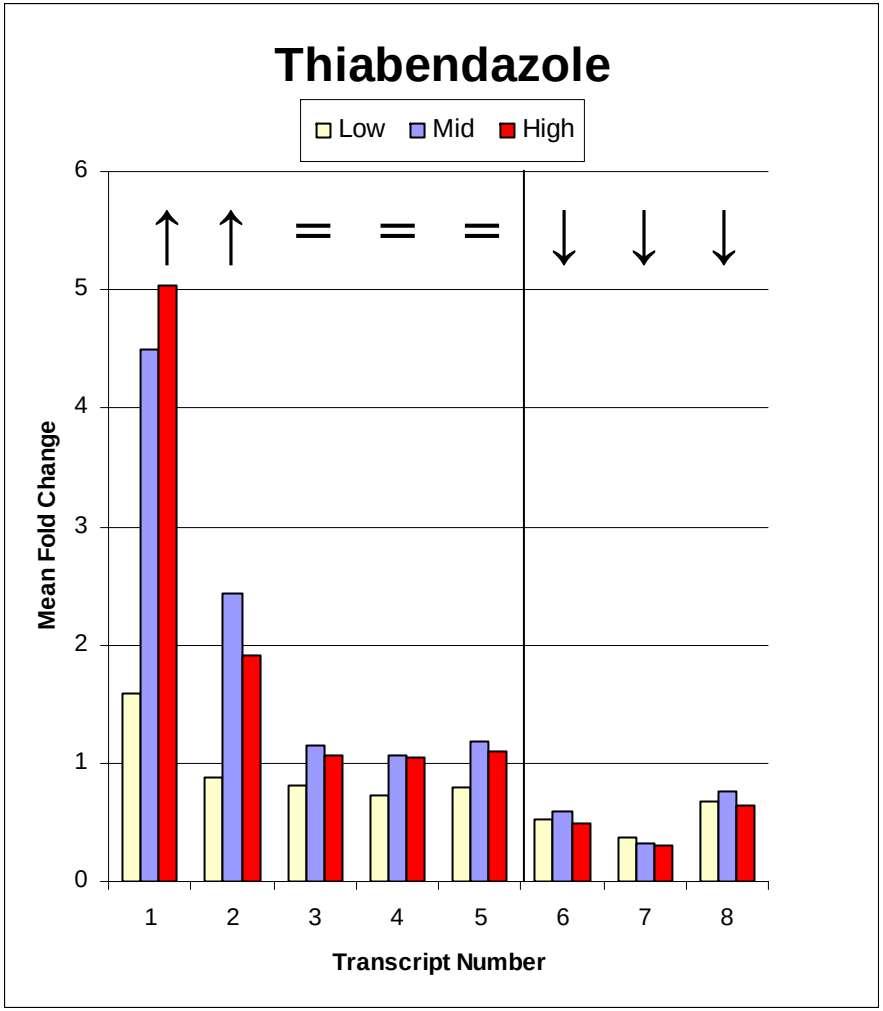
Hepatic Phospholipidosis Subpanel

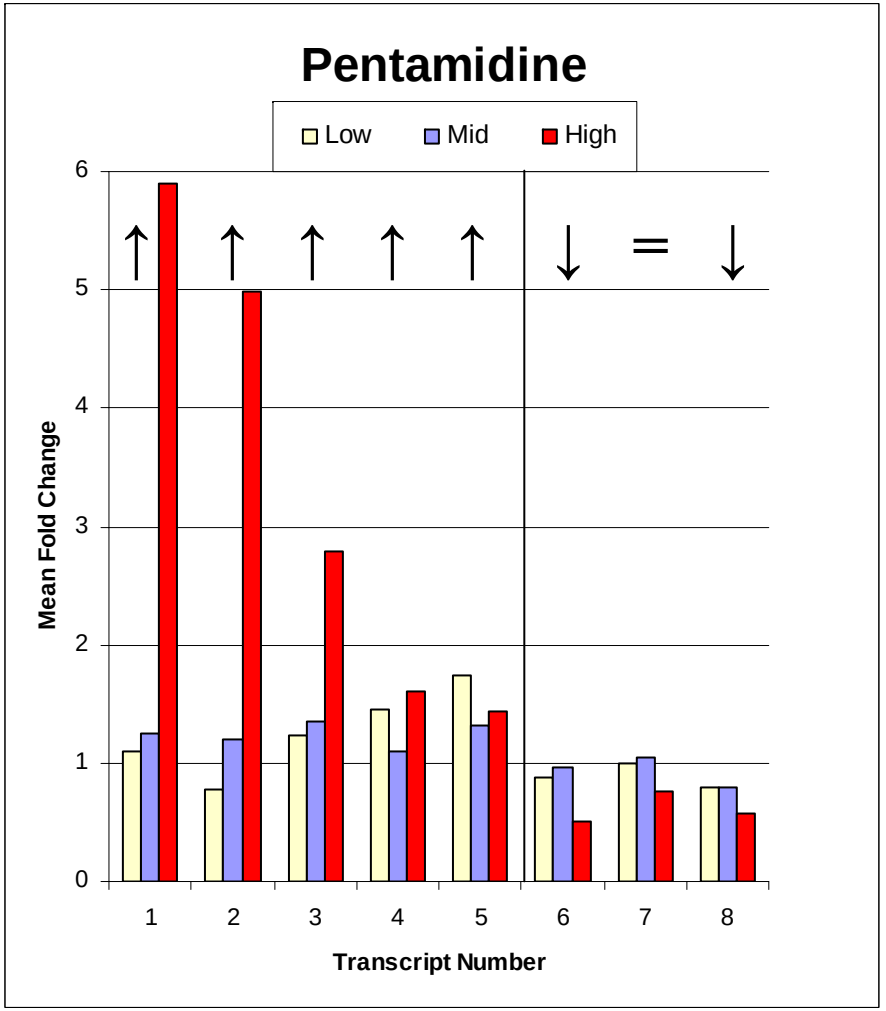
Thiabendazole

Dose-dependent panlobular HC hypertrophy and vacuolation at all doses

Pentamidine

1 low dose rat with periportal vacuolation





Phospholipidosis subpanel predictive of pentamidine-induced phospholipidosis in the absence of histopathology

1: Pharmacol Toxicol. 1994 Jan;74(1):17-22.

Pentamidine accumulates in rat liver lysosomes and inhibits phospholipid degradation.

Glaumann H, Bronner U, Ericsson O, Gustafsson LL, Rombo L.

Department of Infectious Diseases, Karolinska Institute, Sweden.

The subcellular distribution and the effects of pentamidine on the ultrastructure of the rat liver were studied. Rats were given single or repeated daily intraperitoneal injections of 10, 25 or 50 mg pentamidine isethionate/kg b. wt. for 1, 4, 6, 9 or 16 days. The livers were removed for ultrastructural and biochemical analyses on the day after termination of each series of injections and in addition 7 and 35 days after the 16th injection. Electron microscopy of liver tissues showed that the general cellular architecture of the hepatocytes was preserved. The subcellular organelles were normal, except for the secondary lysosomes, which were severely altered and laden with multilamellar, myelin structures (myelin bodies) that gradually increased with dose and time course following repeated injections. These altered lysosomes were enriched in phospholipids. The alteration of the lysosomes persisted for up to 5 weeks after cessation of administration. Pentamidine was highly enriched in the lysosomal fraction (30-50 times more than in the liver homogenate). It was calculated that the lysosomal pentamidine accounted for practically all pentamidine distributed to the liver. The demonstrated accumulation of pentamidine in the lysosomes may explain the known large volume of distribution of this drug and may be one mechanism for organ toxicity.

PMID: 8159632 [PubMed - indexed for MEDLINE]

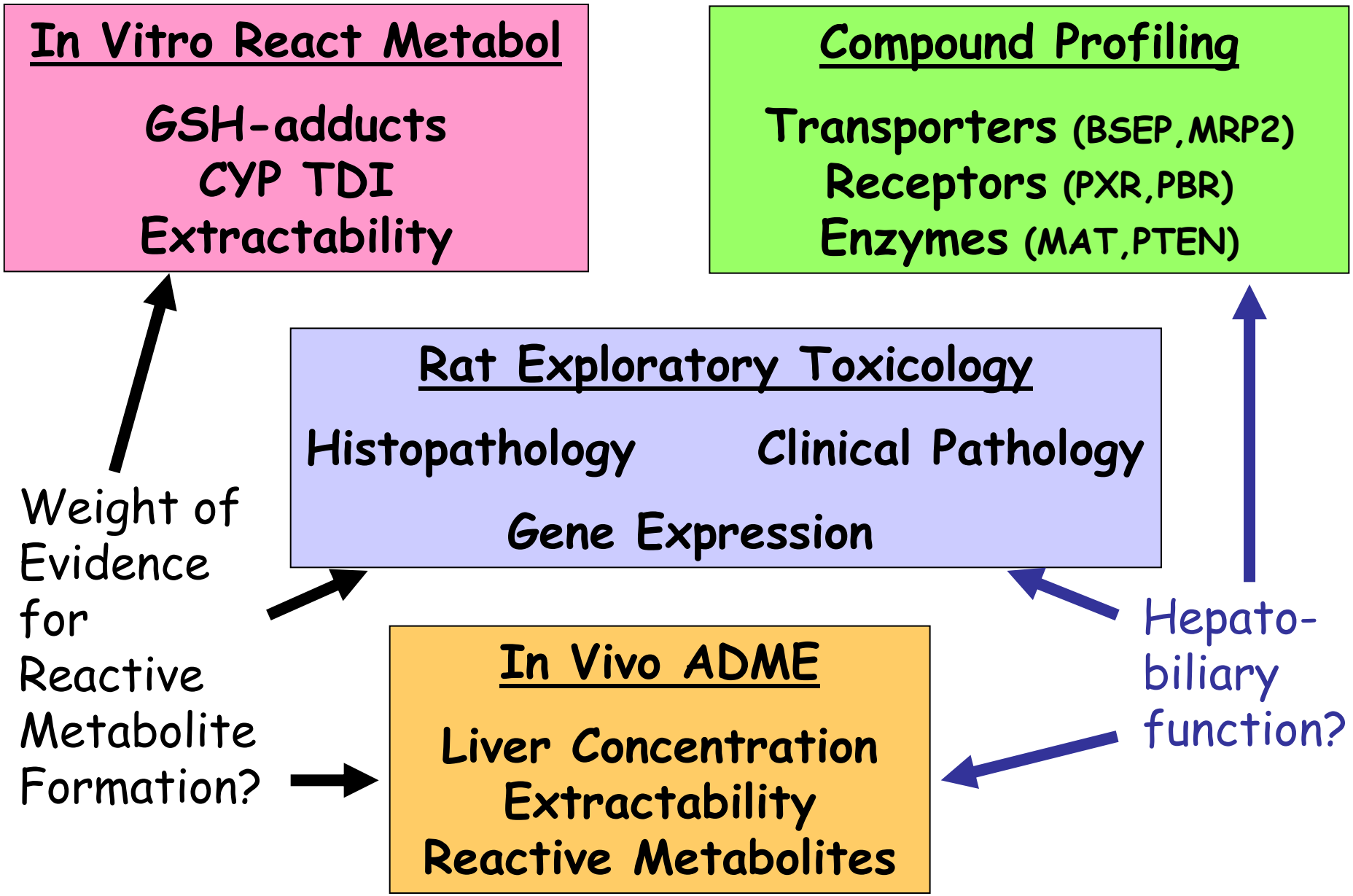
Implementation Strategy

- Apply to rat candidate selection toxicology studies to improve early, toxicological characterization of compounds
  - Precedent for TaqMan™ already existed (P450s)
  - Small, nonGLP study
  - Study objectives and regulatory status (not used for human safety decisions) amenable to exploratory data
  - Presents greatest compound diversity to assess utility
  - Practical integration of toxicogenomics with clinical and morphologic pathology in an established study

Summary

- Identified sets of genes associated with rat liver toxicity modes and manifestations
- Interpret by comparison to "typical toxicants"
- Attempting prospective validation by collecting data on novel compounds before definitive toxicology or clinical studies
- Gene expression is one type of data used in conjunction with other data for compound characterization to assist candidate selection

Future Challenge: Data Integration



Acknowledgements

Kim Roland, Krista Stayer, Daniela Ennulat, Mark Tirmenstein, Roger Brown, Jeffrey Ambroso, Holly Jordan, Chandi Elangbam, Gianni Dal Negro, Federica Crivellente, Lucinda Weir, Helen Billings, Sarah Nesfield, Maria Beaumont, Paul Trennery Michael Santostefano, Steve Clark, KB Tan, Ryan Boyle, Yifen Chen, Jessica Schreiter, Mike Trower, Mary Brawner, Georgina Paolazzo, Melissa Bertraiux, Kevin Kershner, Jessica Shroeck, Elizabeth Docherty, Derk Bergsma, Sujoy Ghosh, Qi Wang, Klaudia Steplewski, Erin Sharpe, Julie Keller, Ashley Hughes, Emma Akuffo, Jeff Hill, Paul Cutler, Isro Gloger, Louisa Bill, Mike Lutz, Patrick Warren, Mike Lonetto, Jacob Angert, Kay Tatsuoka, Michal Magid-Slav, Junping Jing, Hannah Muthyala, Mike Italia, JoAnn Betts, Leli Sarov-Blat, Marian Birkeland, Dilip Rajagopalan, Prakash Dev, Dave Mack, Edit Kurali, Christine Debouck, David Searls

gsk GlaxoSmithKline